CLAIMS

1. A process that simultaneously detects methylation at multiple CpG island sites using a reference sample obtained from a sample to be tested, wherein the process is a nucleic acid methylation detection process that uses an internal reference sample and comprises the steps of:

using a DNA sample for analysis, that is divided into a first DNA sample to be tested and a second DNA sample to be the internal reference, to amplify the second DNA sample such that methylcytosine residues are amplified as unmethylated cytosine residues;

converting the unmethylated cytosine residues to deoxyuracil residues in both the first DNA sample and the second DNA sample;

using a first fluorescent marker and a second fluorescent marker having non-overlapping fluorescent excitation and fluorescent emission spectra to label the first DNA sample with the first fluorescent marker and to label the second DNA sample with the second fluorescent marker; and

hybridizing the first DNA sample and the second DNA sample onto a microarray device having a plurality of oligonucleotide capture probes designed to hybridize to CpG island sites of the DNA sample as converted and non-converted forms.

2. A process that simultaneously detects methylation at a large number of CpG island sites using a reference sample obtained from a sample to be tested, comprising:

(a) providing a DNA sample for analysis;

(b) dividing the DNA sample into a first DNA sample and a second DNA sample, whereby the first sample will become a test sample and the second sample will become an internal reference sample;

(c) amplifying the second DNA sample by a nucleic acid amplification process such that methylcytosine residues are amplified as unmethylated cytosine residues;

(d) bisulfite conversion of unmethylated cytosine residues

into deoxyuracil residues in both the amplified first DNA sample and the second DNA sample;

(e) amplifying the converted first DNA sample and the converted second DNA sample;

(f) labeling the bisulfite-converted second DNA sample with a second fluorescent marker and the bisulfite-converted first DNA sample with a first fluorescent marker, wherein the first and second fluorescent markers have non-overlapping fluorescent excitation and emission spectra; and

(g) hybridizing the first DNA sample and the second DNA sample onto a microarray device having a plurality of oligonucleotide capture probes designed to hybridize to CpG island sites of the DNA sample as converted and non-converted by bisulfite.

3. The process of claim 1 or 2, wherein the amplification technique employed is PCR (polymerase chain reaction).

4. The process of any one of claims 1 to 3, wherein the hybridization conditions are highly stringent conditions.

5. The process of any one of claims 1 to 4, wherein the non-overlapping fluorescent labels are Cy3, (1,1'- bis (ε-carboxypentyl) -1'ethyl-3,3,3',3'-tetramethylindocarbocyanine-5,5'-disulfonate potassium salt di-N-hydroxysuccinimide ester) and Cy5 (1,1'-bis(ε-carboxypentyl)-1'ethyl-3,3,3',3'-tetramethylindodicarbocyanine-5,5'-disulfonate potassium salt di-N-hydroxysuccinimide ester).

6. A microarray plate for detecting methylation at cytosine sites in CpG islands in a DNA sample to be tested, on which plate the following oligonucleotides are immobilized:

(a) an oligonucleotide comprising a sequence complementary to a DNA fragment comprising cytosine sites to be tested in the DNA sample, wherein cytosine sites other than the cytosine sites to be tested are substituted with thymines; and

(b) an oligonucleotide comprising a sequence complementary to a DNA fragment comprising cytosine sites to be tested in the DNA sample, wherein all the cytosine sites are substituted with thymines.

7. A kit for detecting methylation at cytosine sites in CpG islands in a DNA sample to be tested, which comprises:

(a) the microarray plate of claim 6,

(b) reagents for bisulfite-conversion and/or DNA labeling reagents.

8. A kit for detecting methylation at cytosine sites in CpG islands in a DNA sample to be tested, which comprises:

(a) an oligonucleotide comprising a sequence complementary to a DNA fragment comprising cytosine sites to be tested in the DNA sample, wherein cytosine sites other than the cytosine sites to be tested are substituted with thymines; and

(b) an oligonucleotide comprising a sequence complementary to a DNA fragment comprising cytosine sites to be tested in the DNA sample, wherein all the cytosine sites are substituted with thymines.